

1 GC CGT CACTCC CTC CGT CAT CGATAACAT CCT GTC CAA GAT CGA GAA CGA GTC CG
 1▶ Al a Val Thr Pro Ser Val Ile Asp Asn Ile Leu Ser Lys Ile Gl u Asn Gl u Tyr G
 56 AGGT GCT GTA CCT GAA GGC CTT GGCAGG GGT CTA CCG GAG CTT GAA GAA GCA G
 19▶ I u Val Leu Tyr Leu Lys Pro Leu Ala Gl y Val Tyr Arg Ser Leu Lys Lys Gl n
 109 CT GGA GAA CAACGT GAT GAC CTT CAA CGT GAA CGT GAAGGATAT CTT GAA CAGC
 37▶ Leu Gl u Asn Asn Val Met Thr Phe Asn Val Asn Val Lys Asp Ile Leu Asn Ser
 163 CG GTT CAA CAA GCG GGA GAA CTT CAA GAA CGT CTT GGA GAGC GAT CTT GAT CCG
 55▶ Arg Phe Asn Lys Arg Gl u Asn Phe Lys Asn Val Leu Gl u Ser Asp Leu Ile Pr
 216 CTA CAA GGAT CTT GAC CAG CAG CAA CTA CGT GGT CAA GGATCC CTA CAA GTT CCG
 72▶ o Tyr Lys Asp Leu Thr Ser Ser Asn Tyr Val Val Lys Asp Pro Tyr Lys Phe L
 269 T GAA CAA GGA GAA GAGAGATAA GTTC CTT GAGCAGTTA CAA CTA CAT CAAGGATAG
 90▶ eu Asn Lys Gl u Lys Arg Asp Lys Phe Leu Ser Ser Tyr Asn Tyr Ile Lys Asp Se
 324 CAT TGATAC CGATAT CAA CTT CCG CAA CGATGT CCT GCGATA CTA CAA GAT CTT
 108▶ r Ile Asp Thr Asp Ile Asn Phe Al a Asn Asp Val Leu Gl y Tyr Tyr Lys Ile Le
 378 GTCCGA GAA GTA CAA GAGC GAT CTT GAT TCAAT CAA GAA GTA CAT CAACGA TAA
 126▶ u Ser Gl u Lys Tyr Lys Ser Asp Leu Asp Ser Ile Lys Lys Tyr Ile Asn Asp Ly
 432 GCA GCG AGA GAA CGAGAA GTAC CTT GCCCTT CTT GACAA CAT CGAGACC CTT GTA
 44▶ s Gl n Gl y Gl u Asn Gl u Lys Tyr Leu Pro Phe Leu Asn Asn Ile Gl u Thr Leu Ty
 486 CAA GAC CGT CAA CGATAA GATTGAT CTT GTT CGT GAT CCA CTT GGA GGC CAA GGT
 162▶ r Lys Thr Val Asn Asp Lys Ile Asp Leu Phe Val Ile His Leu Gl u Al a Lys Va
 NdeI
 540 CCT GAA CTA CACATATGAGAA GAGC AACGT GGA GGT CAA GAT CAA GGA GCT GAA
 80▶ I Leu Asn Tyr Thr Tyr Gl u Lys Ser Asn Val Gl u Val Lys Ile Lys Gl u Leu As
 594 TTAC CTT GAA GAC CAT CCA GGA TAA CTT GGC CGATTT CAA GAA GAA CAACAA CTT
 198▶ n Tyr Leu Lys Thr Ile Gl n Asp Lys Leu Al a Asp Phe Lys Lys Asn Asn Asn Ph
 648 CGT CCG GAT CCG CGAT CTT GAGC AC CGATTA CAACCA CAA CAAC CTT CTT GAC CAA
 216▶ e Val Gl y Ile Al a Asp Leu Ser Thr Asp Tyr Asn His Asn Asn Leu Leu Thr Ly
 702 GTTCCT GAG CAC CGGTATGGT CTT CGAAAA CTT GGC CAA GACCGT CTT GAGC AA
 234▶ s Phe Leu Ser Thr Gly Met Val Phe Gl u Asn Leu Al a Lys Thr Val Leu Ser As
 756 CTT GCT GGATGG GAAC CTT GCA GCG GATG CTT GAACAT CAGC CA GCACCA GTG TGT
 252▶ n Leu Leu Asp Gl y Asn Leu Gl n Gly Met Leu Asn Ile Ser Gl n His Gl n Cys Va
 810 GAA GAA GCA GTGTCC CCA GAA CAGC GGT GTGTTTCAGACA CTT GGATGA GAGAGA
 270▶ I Lys Lys Gl n Cys Pro Gl n Asn Ser Gl y Cys Phe Arg His Leu Asp Gl u Arg Gl
 864 GGA GTGTAA GTGT CTT CTT GAA CTACAA GCA GGAAGGTGATAA GTGTGT GGA AAAC
 288▶ u Gl u Cys Lys Cys Leu Leu Asn Tyr Lys Gl n Gl u Gl y Asp Lys Cys Val Gl u Asn
 919 CC CAATCCTACTTGTAACGA GAA CAATGGTGGATGTGATGC CGATGCCAA GTGTACCG
 307▶ Pro Asn Pro Thr Cys Asn Gl u Asn Asn Gly Gl y Cys Asp Al a Asp Al a Lys Cys Thr G
 977 A GGA GGATTCAGG GAGCAACGG GAAGAA GATCAC CTTGTGA GTGTAC CAA CCTGATT
 326▶ I u Gl u Asp Ser Gl y Ser Asn Gl y Lys Lys Ile Thr Cys Gl u Cys Thr Lys Pro Asp S
 1034 CTTATCCACT GTTCGATGGTAT CTTCTG TAGT
 345▶ er Tyr Pro Leu Phe Asp Gl y Ile Phe Cys Ser

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F16.2

EcoNI (73)

56 AGGTTTTATATTTAAACCTTTAGCAGGTGTTTATAGAAGTTTAAAAAACAAATT
19▶ IuValLeuTyrLeuLysProLeuAlaGlyValTyrArgSerLeuLysLysGlnLe
111 AGAAAATAACGTTATGACATTTAATGTTAATGTTAAGGATATTTTAAATTCACGA
37▶ uGluAsnAsnValMetThrPheAsnValAsnValLysAspIleLeuAsnSerArg
166 TTTAATAAACGTGAAAATTTCAAAAATGTTTTAGAATCAGATTTAATTCATATA
56▶ PheAsnLysArgGluAsnPheLysAsnValLeuGluSerAspLeuIleProTyrL
221 AAGATTTAACATCAAGTAATTATGTTGTCAAAGATCCATATAAATTTCTTAATAA
74▶ ysAspLeuThrSerSerAsnTyrValValLysAspProTyrLysPheLeuAsnLy
276 AGAAAAAGAGATAAATTCCTTAAGCAGTTATAATTTATATTAAGGATTCAATAGAT
92▶ sGluLysArgAspLysPheLeuSerSerTyrAsnTyrIleLysAspSerIleAsp
331 ACGGATATAAATTTTGCAAATGATGTTCTTGGATATTATAAAATATTATCCGAAA
111▶ ThrAspIleAsnPheAlaAsnAspValLeuGlyTyrTyrLysIleLeuSerGlu
386 AATATAAATCAGATTTAGATTCAATTAAAAAATATATCAACGACAAACAAGGTGA
129▶ ysTyrLysSerAspLeuAspSerIleLysLysTyrIleAsnAspLysGlnGlyGlu
441 AAATGAGAAATACCTTCCCTTTTTAAACAATATTGAGACCTTATATAAAACAGTT
147▶ uAsnGluLysTyrLeuProPheLeuAsnAsnIleGluThrLeuTyrLysThrVal
496 AATGATAAAATTGATTTATTGTGAATTCATTTAGAAGCAAAAGTTCTAAATTATA
166▶ AsnAspLysIleAspLeuPheValIleHisLeuGluAlaLysValLeuAsnTyrT
551 CATATGAGAAATCAAAACGTAGAAGTTAAAATAAAAGAACTTAATTACTTAAAAAC
184▶ hrTyrGluLysSerAsnValGluValLysIleLysGluLeuAsnTyrLeuLysTh
606 AATTCGAAGACAAATTGGCAGATTTTTAAAAAAAATAACAATTTTCGTTGGAATTGCT
202▶ rIleGluAspLysLeuAlaAspPheLysLysAsnAsnAsnPheValGlyIleAla
551 GATTTATCAACAGATTATAACCATAATAAATTATTGACAAAGTTCCTTAGTACAG
221▶ AspLeuSerThrAspTyrAsnHisAsnAsnLeuLeuThrLysPheLeuSerThrG
216 GTATGGTTTTTGAATCTTGCTAAAACCGTTTTATCTAATTTACTTGATGGA
239▶ IyMetValPheGluAsnLeuAlaLysThrValLeuSerAsnLeuLeuAspGlyAs
271 CTTCGAAGGTATGTTAAACATTTCAACACCAATCGGTAAAAAACAAATGTCCA
257▶ nLeuGlnGlyMetLeuAsnIleSerGlnHisGlnCysValLysLysGlnCysPro
266 CAAAATTCCTGGATGTTTCAGACATTTAGATGAAAGAGAAGAATGTAAATGTTTAT
276▶ GlnAsnSerGlyCysPheArgHisLeuAspGluArgGluGluCysLysCysLeuL
281 TAAATTTACAACAAGAAGGTGATAAATGTGTTGAAAATCCAAATCCTACTTGTAA
294▶ euAsnTyrLysGlnGluGlyAspLysCysValGluAsnProAsnProThrCysAs
336 CGAAAATAATGGTGGATGTGATGCAGATGCCAAATGTACCGAAGAAGATTCAGGT
12▶ nGluAsnAsnGlyGlyCysAspAlaAspAlaLysCysThrGluGluAspSerGly
991 AGCAACGGAAGAAAATCACATGTGAATGTACTAAACCTGATTCTTATCCACTTT
331▶ SerAsnGlyLysLysIleThrCysGluCysThrLysProAspSerTyrProLeuP

1046 TCGATGGTATTTTCTGCGATCACCACCACCACCACCTAACT
349► heAspGly l l ePheCysSer Hi sHi sHi sHi sHi s • • •

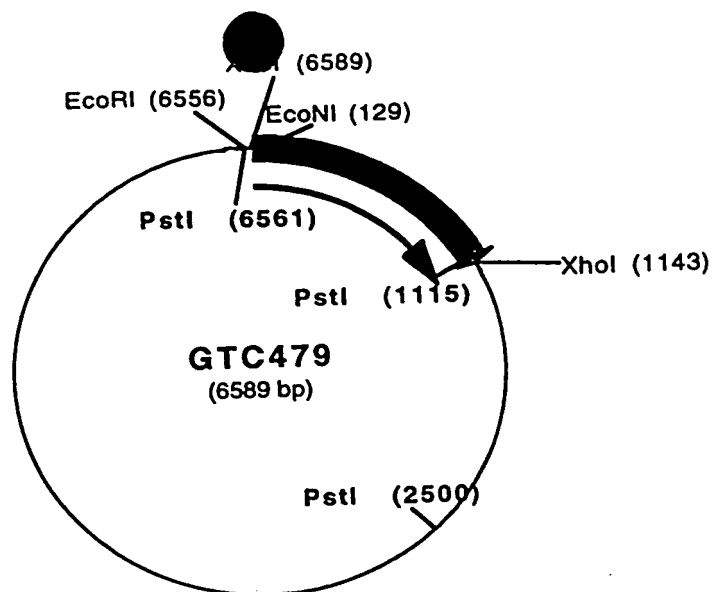
FIG. 3A

Codon	AA	goat b-casein	goat K-casein	MSP wt	Edited MSP	mouse b-casein	mouse a-casein	mouse y-casein	mouse e-casein
TTT	Phe	5	4	8	0	4	8	3	4
TTC	Phe	4	3	7	15	4	6	7	1
TTA	Leu	0	2	25	0	0	0	0	0
TTG	Leu	0	2	3	0	0	0	0	1
TCT	Ser	5	1	4	1	13	5	7	5
TOC	Ser	2	2	2	3	6	14	8	2
TCA	Ser	1	4	10	1	1	3	2	0
TOG	Ser	0	1	0	0	0	0	0	0
TAT	Tyr	2	7	17	2	1	3	2	1
TAC	Tyr	1	2	3	18	2	6	6	7
TAA	...	1	2	0	0	1	0	1	0
TAG	...	0	0	0	0	0	0	0	0
TGT	Cys	1	1	10	12	0	0	1	0
TGC	Cys	0	2	2	0	2	2	2	1
TGA	...	0	0	0	0	0	1	0	1
TGG	Trp	1	1	0	0	0	2	2	2
CTT	Leu	9	1	9	0	16	9	3	3
CTC	Leu	5	2	0	0	7	8	0	1
CTA	Leu	1	2	1	0	1	2	1	0
CTG	Leu	11	5	0	38	10	17	4	1
CCT	Pro	17	6	4	2	8	6	3	0
CCC	Pro	12	0	1	6	8	6	6	4
CCA	Pro	3	13	5	1	5	6	2	2
CCG	Pro	1	1	0	1	0	0	0	1
CAT	His	0	1	3	0	2	6	2	1
CAC	His	5	3	1	4	4	0	3	0
CAA	Gln	5	9	9	0	9	21	9	7
CAG	Gln	16	6	0	9	21	32	12	8
CGT	Arg	0	1	1	0	0	0	0	0
CGC	Arg	0	0	0	0	1	0	0	0
CGA	Arg	0	0	1	0	0	0	0	1
CGG	Arg	1	0	0	3	0	0	0	0
ATT	Ile	4	5	13	0	3	4	3	4
ATC	Ile	6	3	2	20	7	5	8	5
ATA	Ile	1	3	5	0	1	0	2	0
ATG	Met	7	3	3	3	4	12	2	13
ACT	Thr	7	6	3	2	6	5	1	4
ACC	Thr	2	7	3	13	4	4	4	4
ACA	Thr	2	4	9	1	1	1	2	0
ACG	Thr	0	0	1	0	0	0	2	0
AAT	Asn	2	6	29	3	4	6	3	1
AAC	Asn	2	3	12	38	4	9	4	6
AAA	Lys	7	6	38	0	6	7	3	5
AAG	Lys	6	4	4	42	3	6	13	7
AGT	Ser	2	6	5	2	3	6	6	5
AGC	Ser	5	0	2	16	2	6	6	3
AGA	Arg	2	2	4	3	1	8	1	1
AGG	Arg	0	2	0	0	0	0	0	1
GTT	Val	5	6	15	0	7	4	2	3
GTC	Val	8	2	1	11	7	3	3	0
GTA	Val	2	2	5	0	2	4	1	3
GTG	Val	8	4	0	10	6	3	5	3
GCT	Ala	1	3	2	0	8	17	4	2
GCC	Ala	4	7	1	8	6	3	3	3
GCA	Ala	3	7	6	1	4	13	1	1
GCG	Ala	0	1	0	0	0	0	0	0
GAT	Asp	4	5	25	27	3	6	4	2
GAC	Asp	0	2	2	0	1	2	1	3
GAA	Glu	10	6	21	3	6	12	9	6
GAG	Glu	9	5	4	22	5	5	5	5
GGT	Gly	2	1	8	4	0	0	0	0
GGC	Gly	0	0	0	0	0	0	0	0
GGA	Gly	2	1	6	3	1	0	1	0
GGG	Gly	1	0	0	7	1	0	0	0

FIG.3B

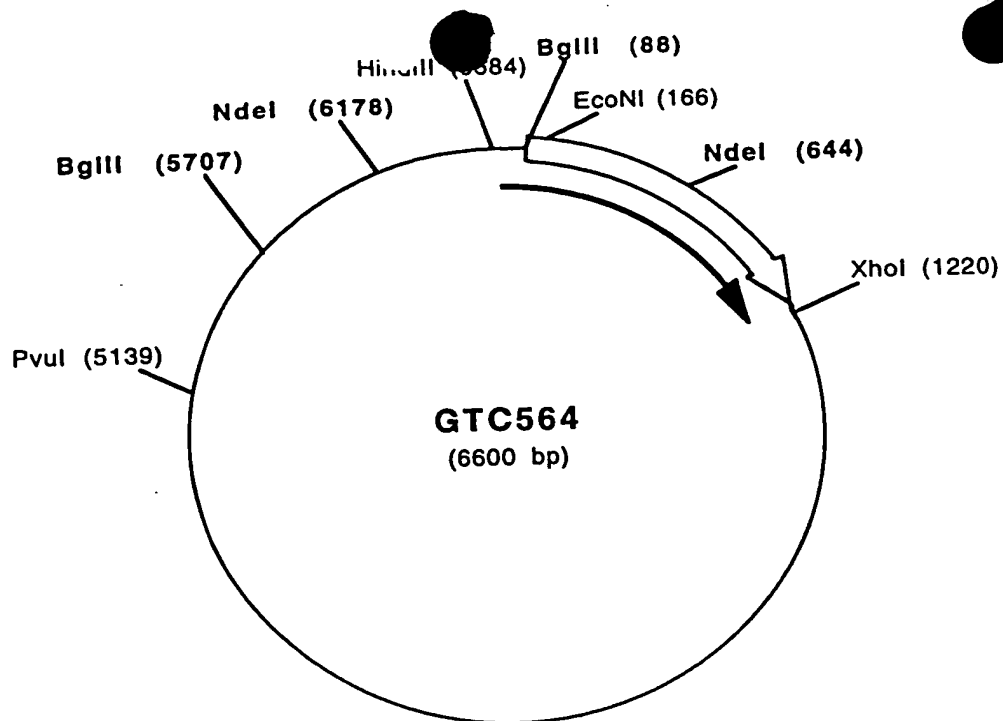
Codon	AA	MSP wt	Edited MSP	MSP wt	Edited MSP	E.coli	Human
TTT	Phe	8	0	0.53	0	0.5	0.35
TTC	Phe	7	15	0.47	1	0.5	0.65
TTA	Leu	25	0	0.66	0	0.11	0.05
TTG	Leu	3	0	0.08	0	0.11	0.09
TCT	Ser	4	1	0.17	0.04	0.27	0.17
TCC	Ser	2	3	0.09	0.13	0.21	0.26
TCA	Ser	10	1	0.43	0.04	0.13	0.11
TCG	Ser	0	0	0	0	0.14	0.07
TAT	Tyr	17	2	0.85	0.1	0.54	0.47
TAC	Tyr	3	18	0.15	0.9	0.46	0.53
TAA	***	0	0				
TAG	***	0	0				
TGT	Cys	10	12	0.83	1	0.45	0.3
TGC	Cys	2	0	0.17	0	0.55	0.7
TGA	***	0	0				
TGG	Trp	0	0	0	0	1	1
CTT	Leu	9	0	0.24	0	0.12	0.11
CTC	Leu	0	0	0	0	0.12	0.22
CTA	Leu	1	0	0.02	0	0.03	0.07
CTG	Leu	0	38	0	1	0.72	0.46
CCT	Pro	4	2	0.4	0.2	0.14	0.24
CCC	Pro	1	6	0.1	0.6	0.11	0.41
CCA	Pro	5	1	0.5	0.1	0.2	0.24
CCG	Pro	0	1	0	0.1	0.54	0.11
CAT	His	3	0	0.75	0	0.64	0.42
CAC	His	1	4	0.25	1	0.36	0.58
CAA	Gln	9	0	1	0	0.31	0.26
CAG	Gln	0	9	0	1	0.69	0.74
CGT	Arg	1	0	0.17	0	0.46	0.09
CGC	Arg	0	0	0	0	0.32	0.19
CGA	Arg	1	0	0.17	0	0.05	0.1
CGG	Arg	0	3	0	0.5	0.06	0.15
ATT	Ile	13	0	0.65	0	0.39	0.23
ATC	Ile	2	20	0.1	1	0.52	0.64
ATA	Ile	5	0	0.25	0	0.08	0.13
ATG	Met	3	3	1	1	1	1
ACT	Thr	3	2	0.19	0.13	0.36	0.2
ACC	Thr	3	13	0.19	0.81	0.38	0.47
ACA	Thr	9	1	0.56	0.06	0.09	0.21
ACG	Thr	1	0	0.06	0	0.17	0.12
AAT	Asn	29	3	0.71	0.07	0.29	0.34
AAC	Asn	12	38	0.29	0.93	0.71	0.66
AAA	Lys	38	0	0.9	0	0.72	0.45
AAG	Lys	4	42	0.1	1	0.28	0.55
AGT	Ser	5	2	0.21	0.09	0.11	0.11
AGC	Ser	2	16	0.09	0.7	0.14	0.29
AGA	Arg	4	3	0.67	0.5	0.08	0.24
AGG	Arg	0	0	0	0	0.03	0.23
GTT	Val	15	0	0.71	0	0.37	0.13
GTC	Val	1	11	0.05	0.52	0.12	0.27
GTA	Val	5	0	0.24	0	0.28	0.09
GTG	Val	0	10	0	0.48	0.23	0.5
GCT	Ala	2	0	0.22	0	0.33	0.31
GCC	Ala	1	8	0.11	0.89	0.18	0.4
GCA	Ala	6	1	0.67	0.11	0.28	0.17
GCG	Ala	0	0	0	0	0.21	0.12
GAT	Asp	25	27	0.93	1	0.48	0.38
GAC	Asp	2	0	0.07	0	0.52	0.62
GAA	Glu	21	3	0.84	0.12	0.67	0.4
GAG	Glu	4	22	0.16	0.88	0.33	0.6
GGT	Gly	8	4	0.57	0.29	0.46	0.15
GGC	Gly	0	0	0	0	0.4	0.44
GGA	Gly	6	3	0.43	0.21	0.06	0.17
GGG	Gly	0	7	0	0.5	0.08	0.24

FIG. 4A



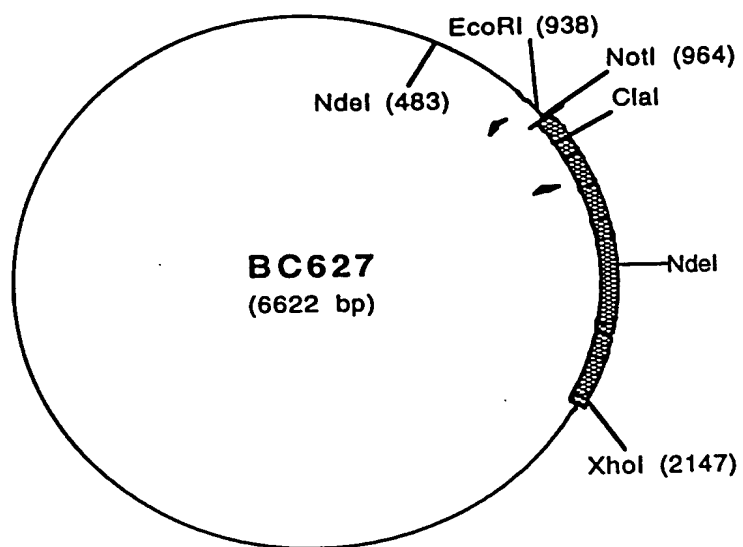
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FIG. 4B



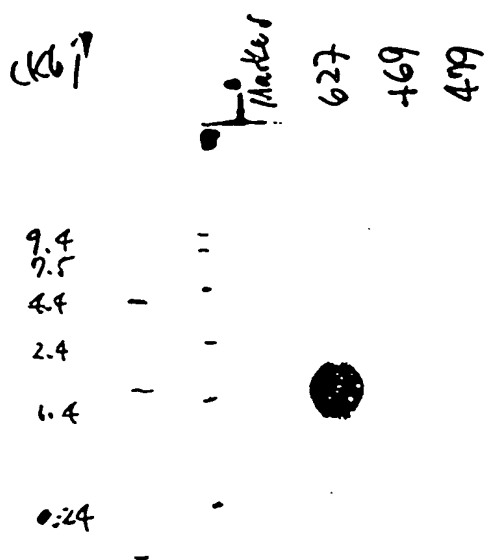
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FIG. 4c

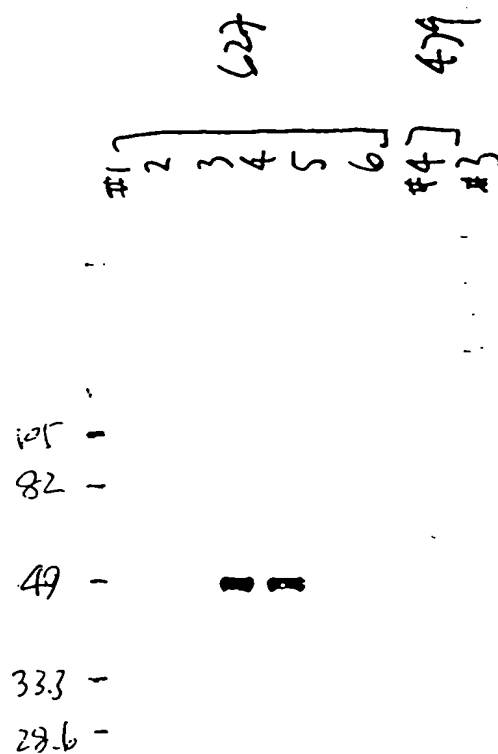


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260207" E2954T60

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Panel A



Panel B

F1C. 6

OT1:
TCG ACG AGA GCC ATG AAG GTC CTC ATC CTT GCC TGT CTG GTG GCT
CTG GCC ATT GCA AGA GAG CAG GAA GAA CTC AAT GTA GTC GGT A,

OT2:
GAT CTA CCG ACT ACA TTG AGT TCT TCC TGC TCT CTT GCA ATG GCC
AGA GCC ACC AGA CAG GCA AGG ATG AGG ACC TTC ATG GCT CTC G,

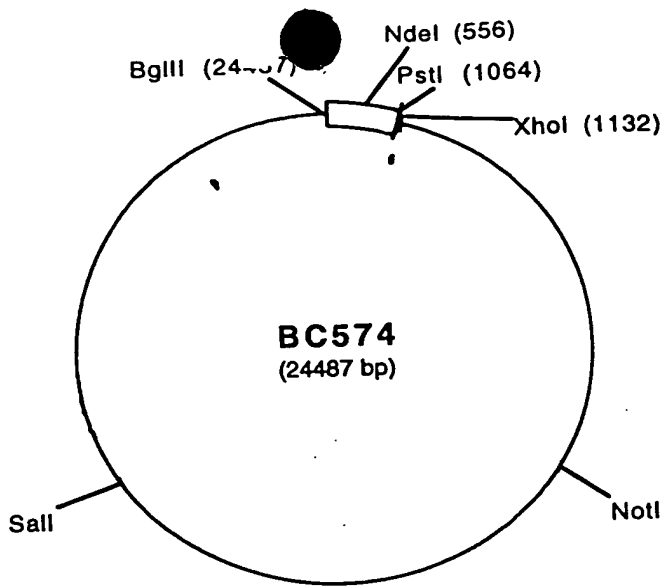
MSP1:
AATAGATCTGCAGTAACTCCTTCCGTAATTG,

MSP2:
AATTCTCGAGTTAGTGGTGGTGGTGGTGGTGA CTGCAGAAATACCATC

MSP8:
TAACTCGAGCGAACCATGAAGGTCCTCATCCTTGCCTGTCTGGTGGCTCTGG
CCATTGCA

091363-1099

FIG. 7



86020T" E895ZT60

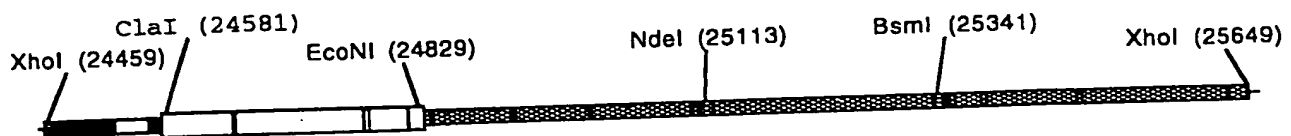


Diagram of BC620

FIGURE 8

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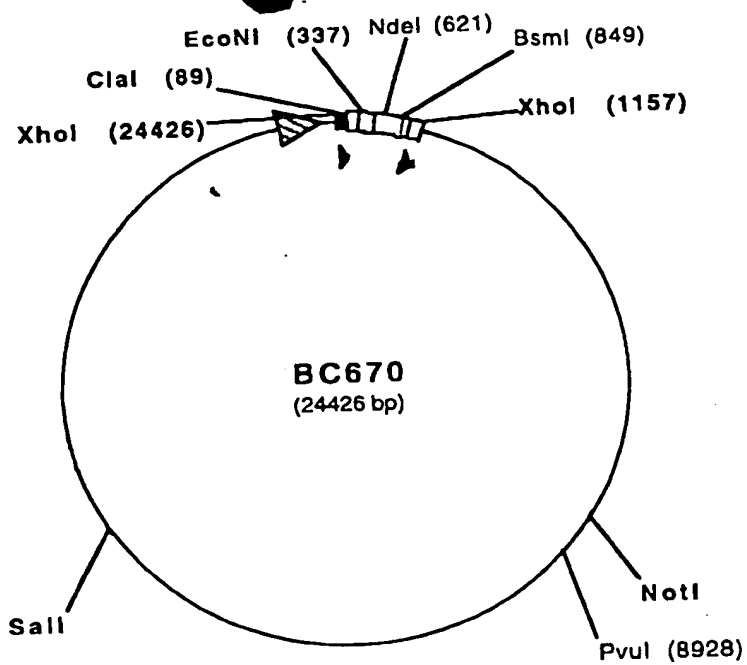
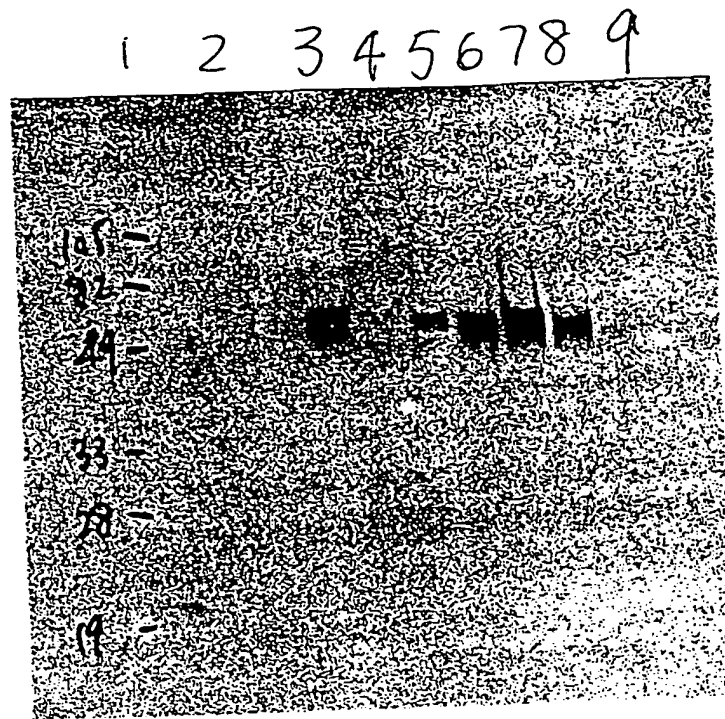


FIGURE 9

09175583 102098

0915563 102098



Western Analysis of MSP transgenic milk.

Lane 1, Molecular weight marker; lane 2, nontransgenic mice milk; lane 3, milk from BC628-146 transgenic mouse; lane 4-9, milk from BC670 transgenic mice. The blot was reacted with monoclonal antibody 5.2 against MSP.

FIGURE 10

26 ATGAAGGTCTCATAATTGCCTGTCTGGTGGCTCTGGCCATTGCAGCCGTCACTCCCTCCGTCATCGATAAC
1 M K V L I I A C L V A L A I A A V T P S V I D N
98 ATCTGTCCAAGATCGAGAAGGATACGAGGTGCTGTACCTGAAGCCCTGGCAGGAGTCTACAAGAGCCT
25 I L S K I E N E Y E V L Y L K P L A G V Y R S L
169 GAAGAAGCAGCTGGAGAACAACGTGATGACCTTCAACGTGAACGTGAAGGATATCTGAACAGCA GGTTCAA
48 K K Q L E N N V M T F N V N V K D I L N S R F N
241 CAAGA GGGAGAACTTCAAGAACGTGCTGGAGAGCGATCTGATCCCTACAAGGATCTGACCAGCAGCAACTA
72 K R E N F K N V L E S D L I P Y K D L T S S N Y
EcoNI (337)
313 CGTGGTCAAGATCCCTACAAGTTCCTGAACAAGGAGAAGAGATAAGTTCTGAGCAGTTACAATTACAT
96 V V K D P Y K F L N K E K R D K F L S S Y N Y I
385 CAAGGATAGCATTGACACCGATATCAACTTCGCCAACCAGTGTCTGGGATACTACAAGATCTGTCCGAGAA
120 K D S I D T D I N F A N D V L G Y Y K I L S E K
457 GTACAAGAGCGATCTGGATAGCATCAAGAGTACATCAACGATAAGCAGGGAGAGAACGAGAAGTACCTGCC
144 Y K S D L D S I K K Y I N D K Q G E N E K Y L P
529 CTTCTGAACAACATCGAGACCCTGTACAAGACCGTCAACGATAAGATTGATCTGTTCGTGATCCACCTGGA
168 F L N N I E T L Y K T V N D K I D L F V I H L E
NdeI (621)
601 GGCCAAGGTCTGCAATACACATATGAGAAGAGCAACCTGGAGGTCAAGATCAAGGAGCTGAATTACCTGAA
192 A K V L Q Y T Y E K S N V E V K I K E L N Y L K
673 CACCATCCAGATAAGCTGGCCGATTTCAGAAGAACAACAACCTTCCTCGGAATCCCGATCTGAGCACCGA
216 T I Q D K L A D F K K N N N F V G I A D L S T D
745 TTACAACCAACAACCTGCTGACCAAGTTCCTGAGCACCGGAATGGTCTTCGAAAACCTGGCCAAGACCGT
240 Y N H N N L L T K F L S T G M V F E N L A K T V
BsmI (849)
817 CCTGAGCAACCTGCTGGATGGAACCTGCAGGGAATGCTGCAATCAACCAGCACCAGTGTGTGAAGAAGC
264 L S N L L D G N L Q G M L Q I S Q H Q C V K K
888 AGTGTCCTCCAGAACAGCGGATGCTTCAGACACCTGGATGAGA GGGAGGAGT GCAAGTGCCTGCTGAACCTA
288 Q C P Q N S G C F R H L D E R E E C K C L L N Y
958 CAAGCAGGAAGGAGATAAGTGTGTGGAACCCCAATCCTACTTGTAAACGAGAACAATG GAGGATGCGATG
311 K Q E G D K C V E N P N P T C N E N N G G C D
1029 CCGATGCCAAGTGTACCGAGGAGGATTGAGGAAGCAACGGAAGAAGATCACCTGCCAGTGTACCAAGCCT
335 A D A K C T E E D S G S N G K K I T C E C T K P
XhoI (1157)
1100 GATTCTTATCCACTGTTTCGATGGTATTTCTGCAGTCACCACCACCACCACCACTAACTCGAGGAT
359 D S Y P L F D G I F C S H H H H H • L E D

FIGURE 11

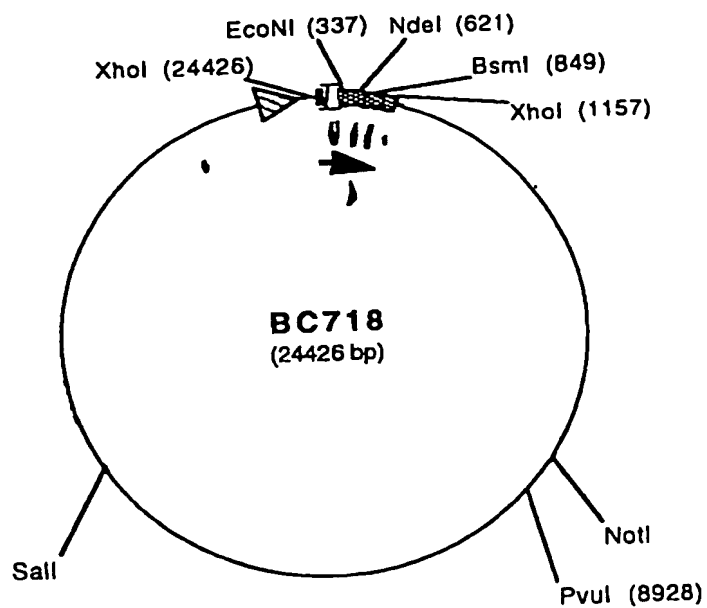


FIGURE 12

860201 " E8952160

FIGURE 13